



UNIVERSITI PUTRA MALAYSIA

**PHYTOCHEMICAL AND BIOLOGICAL ACTIVITY STUDIES OF
HEDYOTIS HERBACEA, *HEDYOTIS DIFFUSA* AND ROOTS OF
*GARCINIA ATROVIRIDIS***

DHARMA PERMANA

FSAS 2002 18

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HEDYOTIS HERBACEA, *HEDYOTIS DIFFUSA* AND ROOTS OF
*GARCINIA ATROVIRIDIS***

By

DHARMA PERMANA

**Thesis Submitted in Fulfilment of the Requirement for the Degree of
Doctor of Philosophy in the School of Graduate Studies
Universiti Putra Malaysia**

January 2002



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Prof. Dr. Nordin Hj Lajis

Faculty : Science and Environmental Studies

A new anthraquinone, 2-hydroxymethyl-10-hydroxy-1,4-anthraquinone (9) was isolated from the dried aerial part of *Hedyotis herbacea* (Rubiaceae) together with five other known compounds, 1,4-dihydroxy-2-hydroxymethylantraquinone (10), 2,3-dimethoxy-9-hydroxy-1,4-anthraquinone (11), 1,4-dihydroxy-2,3-dimethoxylantraquinone (12), kaempferol 3-*O*-rutinose (13) and arbutin (14). Extraction and isolation of the constituents from *Hedyotis diffusa* has yielded three compounds, quercetin 3-*O*-glucose (15), quercetin 3-*O*-rutinose (16) and asperuloside (17). Three new prenylated compounds, the depsidone atrovirisidone (24), the benzoquinone atrovirinone (25) and the hydroquinone 4-methyl-dihydroatrovirinone (26), along with three known compounds, 14-*cis*-docosenoic acid (27), morelloflavone (28) and fukugiside (29) have been isolated from the ethyl

acetate fraction of the methanol crude extract of the dried roots of *Garcinia atroviridis*.

In the biological activity assay, quercetin 3-*O*-glucose (**15**), quercetin 3-*O*-rutinose (**16**), atrovirisidone (**24**), morelloflavone (**28**) and fukugiside (**29**) were found to have stronger antioxidant activity than vitamin E. 2-hydroxymethyl-10-hydroxy-1,4-anthraquinone (**9**) and quercetin 3-*O*-rutinose (**16**) showed strong toxic activity in the brine shrimp lethality assay with a 50 % lethal concentration of 9.7 µg/mL and 23.4 µg/mL, respectively. Atrovirisidone (**24**) and atrovirinone (**25**) were very effective against the microbe *Bacillus subtilis* (mutant type) and *Bacillus subtilis* (wild type) (MID 10 µg/disc for both). Quercetin 3-*O*-glucose (**15**), quercetin 3-*O*-rutinose (**16**), 4-methyl-dihydroatrovirinone (**26**), morelloflavone (**28**) and fukugiside (**29**) showed weak activity compared to atrovirisidone (**24**) and atrovirinone (**25**) but it is still significantly active (MID 100 µg/disc for all compounds). Only atrovirisidone (**24**) exhibited cytotoxic activity towards HeLa cells at IC₅₀ 15 µg/mL that was comparable to the standards doxorubicin (IC₅₀ 11 µg/mL) and colchicine (IC₅₀ 21 µg/mL).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan ijazah Doktor Falsafah

**KAJIAN KIMIA DAN BIOCERAKINAN DARI TUMBUHAN *HEDYOTIS*
HERBACEA, *HEDYOTIS DIFFUSA* DAN AKAR *GARCINIA ATROVIRIDIS***

Oleh

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Satu antrakuinon baru, 2-hidroksimetil-10-hidroksi-1,4-antrakuinon (9), telah diasingkan daripada *Hedyotis herbacea* (Rubiaceae) bersamaan dengan lima sebatian yang dikenal pasti, 1,4-dihidroksi-2-hidroksimetilantrakuinon (10), 2,3-dimetoksi-9-hidroksi-1,4-antrakuinon (11), 1,4-dihidroksi-2,3-dimetoksilantrakuinon (12), kaempferol 3-*O*-rutinosa (13) dan arbutin (14). Pengekstrakan dan pengasingan dari *Hedyotis diffusa* telah didapatkan tiga sebatian, quercetin 3-*O*-glukosa (15), quercetin 3-*O*-rutinosa (16) dan asperulosida (17). Tiga sebatian terprenil yang baru depsidon atrovirisidon (24), benzokuinon atrovirinon (25) dan hidrokuinon 4-metil-dihidroatrovirinon (26) bersamaan dengan tiga sebatian yang dikenal pasti sebagai asid 14-*sis*-dokosenoik (27), moreloflavon (28) dan fukugisid (29) telah diasingkan daripada fraksi etil asetat bagi ekstrak metanol akar kering *Garcinia atroviridis*.

Dalam kajian biocerakan, sebatian quercetin 3-*O*-glukosa (15), quercetin 3-*O*-rutinosa (16), atrovirisidon (24), moreloflavon (28) dan fukugisid (29) didapati mempamerkan aktiviti anti oksidan yang lebih baik dari Vitamin E. Sebatian 2-hidroksimetil-10-hidroksi-1,4-antrakuinon (9) dan quercetin 3-*O*-rutinosa (16) menunjukkan aktiviti toksik yang kuat dalam cerakan " brine shrimp " dengan 50 % kematian pada kepekatan 9.7 $\mu\text{g}/\text{mL}$ and 23.4 $\mu\text{g}/\text{mL}$. Sebatian atrovirisidon (24), dan atrovirinon (25) sangat berkesan melawan mikroba *Bacillus subtilis* (mutant type) dan *Bacillus subtilis* (wild type) (MID 10 $\mu\text{g}/\text{disk}$ untuk kedua sebatian). Sebatian quercetin 3-*O*-glukosa (15), quercetin 3-*O*-rutinosa (16), 4-metil-dihidroatrovirinon (26), moreloflavon (28) dan fukugisid (29) menunjukkan aktiviti yang lemah berbanding atrovirisidon (24) dan atrovirinon (25) tetapi masih aktif (MID 100 $\mu\text{g}/\text{disk}$ untuk setiap sebatian). Hanya sebatian atrovirisidon (24) yang mempamerkan aktiviti terhadap sel HeLa pada IC_{50} 15 $\mu\text{g}/\text{mL}$ yang setanding dengan piawaian doksorubisin (IC_{50} 11 $\mu\text{g}/\text{mL}$) dan kolkisin (IC_{50} 21 $\mu\text{g}/\text{mL}$).

ACKNOWLEDGEMENTS

Glory and praise be to God, the Omnipotent, Omniscient and Omnipresent, for providing me with the strength and perseverance to complete this study.

I am very grateful to my supervisors, Prof. Dr. Nordin Hj Lajis, Prof. Dr. Mawardi Rahmani and Prof. Dr. Abdul Manaf Ali for guidance, comment and support.

I would like to thank The Ministry of Science, Technology, and the Environment of Malaysia for my financial assistantship under the research grant IRPA 03-02-04-0043.

I would also like to thank Prof. Norio Aimi, Assoc.Prof. Hiromitsu Takayama and Dr. Mariko Kitajima at Chiba University, Japan for collaboration and comment.

I would like to thank the staffs and all students Natural Products Laboratory , bung Ghaffar, bung Zainudin, bung Sharin, bung Sara , kak Hadiani, Lim Yang Moi, kak Habsah, kak Rohaya, kak Farida, Jasril, Mukram, Khausik, and Khalid for their support and cooperation.

I certify that an examination has meet on 15th January 2002 to conduct the final examination of Dharma Permana on his Doctor of Philosophy thesis entitled “ Phytochemical and Biological Activity Studies of *Hedyotis herbacea*, *Hedyotis diffusa* and Roots of *Garcinia atroviridis* “ in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows :

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I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



DHARMA PERMANA

Date : 22 April 2002

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LIST OF ABBREVIATIONS

| | |
|---------------------------------|------------------------------------|
| MeOH | : Methanol |
| EtOH | : Ethanol |
| EtOAc | : Ethyl Acetate |
| CHCl ₃ | : Chloroform |
| BuOH | : Butanol |
| DMSO | : Dimethyl sulphoxide |
| TLC | : Thin layer chromatography |
| IC ₅₀ | : 50 % Inhibitory concentration |
| LD ₅₀ | : 50 % Lethal dose |
| MID | Minimum inhibitory dose |
| NA | Nutrient agar |
| HeLa | Human cervical carcinoma cell line |
| FTC | Ferric thiocyanate |
| DPPH | 1,1-diphenyl-2-picrylhydrazyl |
| CC | Column chromatography |
| FeCl ₃ | Ferric chloride |
| Mg | Magnesium |
| HCl | Acid chloride |
| KBr | Potassium bromide |
| Na ₂ CO ₃ | Sodium carbonate |

CaCl_2 : Calcium chloride

NaSO_4 : Sodium sulfate

NaCl : Sodium chloride

PART I
THE CONSTITUENTS OF *HEDYOTIS HERBACEA*
AND *HEDYOTIS DIFFUSA*.

INTRODUCTION

Malaysia is very rich with sources of medicinal plants from its tropical forest. The use of plants as medicinal have been well documented by Burkill (1966). *Hedyotis* Linn, a genus of small herbs of the family Rubiaceae commonly found throughout Malaysia. Many species of *Hedyotis* are used in the Malay and Chinese traditional medicines for various purposes such as for tonic, and treatment of dysentery, tonsilitis, appendicitis, hepatitis, tumor and snake bite. These herbs are used also as a stimulant of the immune system, promoting the activity of the white blood cells and helping clear infection from the lymph glands (Burkill, 1996 and Chung et al, 1998). It is claimed to be to excellent help the body through illness.

Hedyotis is an erect decumbent or climbing herbs (0.1- 0.3 m tall) which can be found throughout Asia. *Hedyotis* and *Oldenlandia* are normally grouped together by botanists and the only difference is the way the capsule opens. Together these genera are represented by about 180 species of which 35 were identified in Peninsular Malaysia (Burkill, 1996 and Chung et al, 1998).

In an earlier study on the chemical constituents of *Hedyotis herbacea*, we isolated a compound identified as arbutin [hydroquinone-glucoside] (**14**) as the major component of the plant constituent occurring in this species (Lajis, 1996). The compound occurs in high quantity of up to 2% yield from the dried weight. Literature study indicated that arbutin is important ingredient in cosmetics for skin whitening. Found naturally in the leaves of Japanese pears and cowberries, this substance is highly effective in inhibiting melanin activity in the melanin generating cell that cause freckles, stains and facial spots. Shiseido's white Essence, uses arbutin as an active ingredient (Maeda et al, 1991 and Kitamura et al, 1992).

Hedyotis herbacea Linn and *Hedyotis diffusa* Willd. are small straggling herbs commonly found among the weed in vegetable gardens throughout Malaysia. The fresh leaves of *Hedyotis herbacea* are sold locally and used as poultice to improve blood circulation (Burkill, 1996 and Chung et al, 1998). The local Chinese community uses the aerial part of *Hedyotis diffusa*, in the forms of decoction or paste to remedy tonsillitis, boils, appendicitis, dysentery, urethral infection, hepatitis, for treatment of cancer and snake bites (Burkill, 1996 and Chung et al, 1998).

In view of the medicinal values of this genus, a research work was carried out on the two species with the following objectives:

1. To isolate the chemical constituents of *Hedyotis herbacea* and *Hedyotis diffusa*.
2. To elucidate the structure of the compounds isolated using modern

spectroscopic techniques .

3. Feasibility study on the production high yield of Arbutin from *Hedyotis herbacea* .
4. To carry out the bioassay on the on the isolated compounds



Hedyotis herbacea

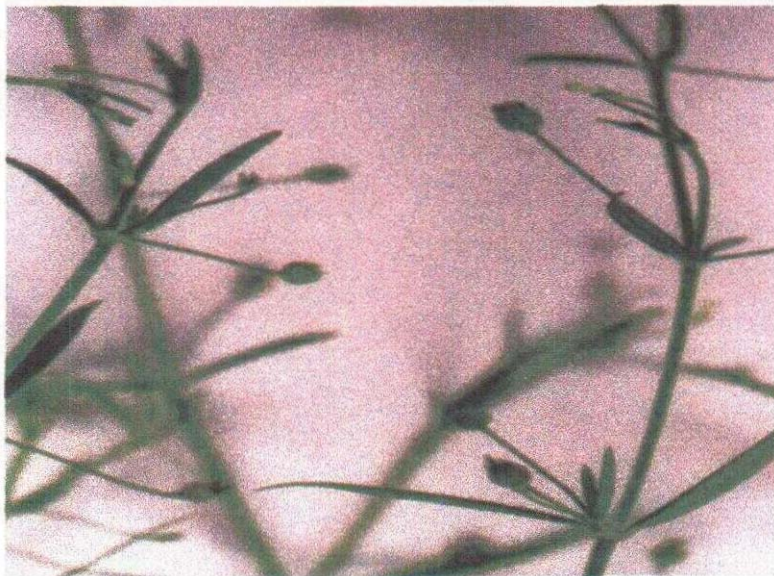


Figure 1: *Hedyotis herbacea* Linn.